

**BIOENERGY PRODUCTION:
BIOHYDROGEN AND ELECTRICITY GENERATION
USING MICROBIAL FUEL CELLS**

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**BACTERIA PRODUCE AND USE REDOX MEDIATORS FOR ELECTRON
TRANSFER IN MICROBIAL FUEL CELLS**

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During a three months enrichment procedure in a glucose-fed microbial fuel cell, increasing power outputs were obtained (1), with a maximum power output of 431 W / m³ of anode reactor compartment (4,31 W / m²) projected anode surface (2). The microbial fuel cell used comprised two plexiglass reactors separated by a proton exchange membrane. The cathode compartment contained an aerated potassium hexacyanoferrate solution facilitating the electron transfer from the graphite cathode to oxygen.

Microbial community composition present in microbial fuel cells

Fingerprint analysis of the total microbial community showed that *Pseudomonas aeruginosa* was one of the dominant species in the microbial fuel cells. *Pseudomonas aeruginosa* is a facultative anaerobic bacterium, which generally can only use oxygen or nitrate as terminal electron acceptor. Hence, the occurrence of this organism was quite unexpected in the anaerobic conditions of the microbial fuel cells.

Pseudomonas aeruginosa, and several *Pseudomonas* species in general, have been extensively described for their production of phenazine and phenazine derivatives. The regulation of these phenazines is yet unclear, although clear evidence has been provided that they partially function as quorum molecules. However, the most interesting feature of several produced phenazine derivatives is their role as redox mediators. Pyocyanin (Fig. 1), one of the more common phenazine derivatives, is able to generate oxygen radicals within other bacteria, induce host-response in plants and plays a large role in human pathogenicity of the bacterium (3). The role of pyocyanin in transferring electrons from the bacteria towards the electrode was studied.

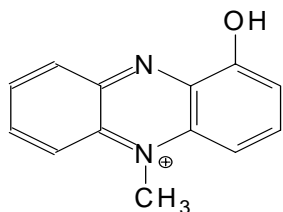


Figure 1. Pyocyanin, a phenazine derivative produced by *Pseudomonas aeruginosa*, which can act as redox mediator

Performance of *Pseudomonas aeruginosa* as pure culture

From the mixed culture, the strain *Pseudomonas aeruginosa* KRP1 was isolated. In a first phase, the growth of the bacterium was examined in microbial fuel cells. As control organisms, a wild type *Pseudomonas aeruginosa* 7NSK2 and the pyocyanin deficient mutant phZ1 of 7NSK2 were used.

The power output of strain KRP1 was significantly higher than strains 7NSK2 and phZ1, namely on average $8.8 \pm 0.3 \text{ W/m}^3$ ($88 \pm 3 \text{ mW/m}^2$) towards $3.1 \pm 0.5 \text{ W/m}^3$ and $4.1 \pm 0.5 \text{ W/m}^3$ (31 ± 5 and $41 \pm 5 \text{ mW/m}^2$) respectively. Bacterial concentrations increased from $1.2 \pm 0.2 \times 10^8 \text{ CFU/ml}$ (equalized for all strains) to $1.8 \pm 0.1 \times 10^9$ for isolate KRP1, $4.4 \pm 0.1 \times 10^8$ for strain 7NSK2, and $6.0 \pm 0.1 \times 10^8$ for strain phZ1. Clearly, the anode isolated strain KRP1 was better adapted to the reactor conditions, resulting in substantially more growth.

The addition of pyocyanin to *Pseudomonas aeruginosa*

Pyocyanin was extracted with chloroform from *Pseudomonas aeruginosa* KRP1 grown on *Pseudomonas* isolation agar and purified. The purity was verified using HPLC.

The pyocyanin was added to a concentration of $50 \mu\text{M}$ to *Pseudomonas aeruginosa* KRP1 in 25 ml anode compartments. This addition resulted in a maximal power output raised from 48 ± 16 to $108 \pm 32 \text{ W/m}^3$, corresponding with an energy increase over the experimental period from 125 ± 62 to $418 \pm 14 \text{ J}$ (Fig. 2). In prior experiments with mixed cultures, the power output always showed a lag phase and then rapidly increased. This increase corresponded to a boost in electrochemical activity as measured by cyclic voltammetry. Clearly, a minimal concentration of pyocyanin is needed to provide sufficient high rate electron transfer. Adding pyocyanin thus improved the electron transfer to a certain extent. However, as reported in literature (4), too high concentrations of pyocyanin ($> 150 \mu\text{M}$) inhibited the bacterial growth.

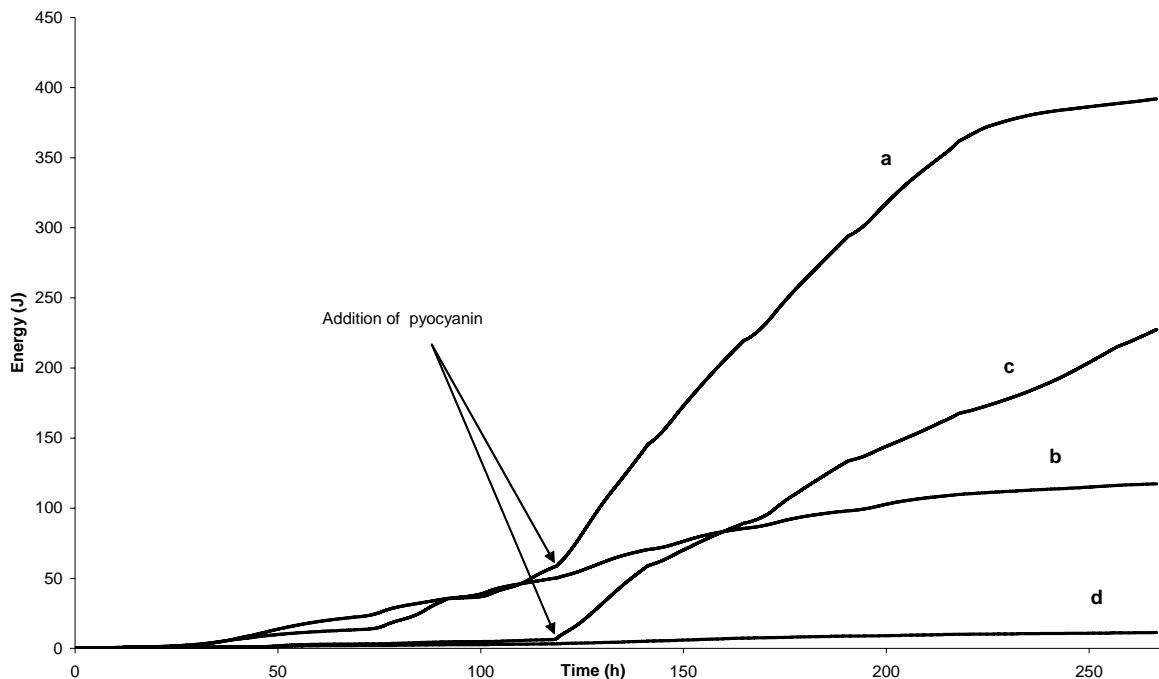


Figure 2. Cumulative energy output of *Pseudomonas aeruginosa* KRP1 (a and b) and *Enterococcus faecium* KRA3. (c and d). Reactors a and c received pyocyanin up to a concentration of 50 μ M at the indicated time and 48 hours later

Induction of the production of mediators

Microbial fuel cells were operated with *Pseudomonas aeruginosa* KRP1 with and without the addition of pyocyanin. In comparison to control reactors, i.e. serum flasks, the production of phenazines was significantly higher in the microbial fuel cells. The addition of pyocyanin did not bring about a higher final phenazine concentration in the reactor. However, microscopic analysis revealed that the bacteria were coloured blue, indicating a sorption onto the membrane or an uptake of the pyocyanin by the bacteria. The bacterial growth was largely affected by the microbial fuel cell conditions. The end concentrations for the MFC grown *Pseudomonas aeruginosa* KRP1 were a factor 3 higher than the concentrations obtained for the control reactors, namely $1,84 \pm 0,38 \times 10^8$ CFU/ml versus $6,47 \pm 1,86 \times 10^7$ CFU/ml.

Use of pyocyanin as redox mediator by other bacteria

Several other isolates from the microbial fuel cell culture were grown in microbial fuel cells and supplemented with 50 μ M pyocyanin. For *Enterococcus faecium* KRA3, nearly no electron transfer was observed without pyocyanin addition. Addition of pyocyanin caused a 23 fold increase of the energy output of the microbial fuel cells. However, when pyocyanin was added to the biofuel cells containing the isolate *Alcaligenes faecalis* KRA1 no effect was observed. Also a lab strain of *Escherichia coli* did not show any difference upon addition of pyocyanin.

Implications and further research

The fact that bacteria produce and use redox mediators in order to reach an alternative electron acceptor, enables them to survive. In case of *P. aeruginosa*, the production of redox mediators enables the bacterium to even gain a numerical dominance within a mixed microbial community.

Several studies performed in the past on 'mediator-less' systems will need re-evaluation. Where mediator-producing bacteria were present, for example in studies with mixed bacterial consortia, the 'mediator less' label should be changed to 'without added mediators'. Theoretical power output calculations need to incorporate the fact that not only a monolayer of bacteria growing onto an electrode can use it as electron acceptor, but also the on-growing bacterial layers, since redox mediators can provide an electric contact between the bacteria and the surface.

The finding that, in a microbial ecosystem, one bacterium can produce a redox mediator that is being used by other bacteria is interesting. This opens perspectives towards the operation of microbial fuel cells: a MFC could be inoculated by a good mediator producer to ensure stable and high levels of energy production by an effective metabolising bacterium.

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